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Beyond biopsy: evaluating noninvasive techniques to diagnose celiac disease in adults

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Abstract

Background Duodenal biopsies are standard for diagnosing celiac disease (CD), but a biopsy-free approach has gained attention in the past decade. Evidence suggests that immunoglobulin A anti-tissue transglutaminase (IgA tTg) antibody levels ≥10 times the upper limit of normal (ULN) may reduce the need for histology. This study aimed to assess whether IgA tTg antibody titers ≥10 × ULN correlate with the histological diagnosis in adults.

Methods The retrospective study was conducted at Mater Dei Hospital, Malta, analyzing adult patients who underwent upper gastrointestinal endoscopy with duodenal biopsies between 2012 and 2024. Data on demographics, symptoms, risk factors, serology and histological results were collected. Patients who had positive serology but initial negative biopsies and underwent repeat biopsies were also reviewed.

Results Of 114 patients (78.1% female, mean age 41.0 years), 97.4% tested positive for IgA tTg antibodies and 93.8% for endomysial antibodies (EMA). CD was histologically confirmed in 70.2%, with females more frequently diagnosed than males (75.3% vs. 52%, P=0.025). CD-related symptoms were reported by 79.8%, while 20.2% were asymptomatic. Levels of tTg ≥10 × ULN were found in 41.2% patients, and this cutoff had a sensitivity of 58.8%, specificity of 100%, positive predictive value of 100% and negative predictive value of 50.7% for CD (P<0.001).

Conclusion This study supports a biopsy-free approach for diagnosing CD when IgA tTg levels are ≥10 x ULN, especially with EMA positivity and typical clinical presentation.

Keywords Celiac disease, biopsy-free, noninvasive, celiac serology

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Introduction

Celiac disease (CD) is a chronic, immune-mediated enteropathy secondary to ingestion of gluten in genetically predisposed children and adults [1]. It can present with a myriad of signs and symptoms, including malabsorption, dermatitis herpetiformis and neurological symptoms such as ataxia. On the other hand, it may be asymptomatic and diagnosed during the screening of at-risk individuals, such as first-degree family members of patients with CD, or those suffering from autoimmune conditions [2,3].

Increased awareness and antibody markers have resulted in a rise in the incidence of CD [1], which highlights the importance of easy, widely available and cost-effective diagnostic tools. Furthermore, since this is a lifelong condition, a highly accurate diagnosis needs to be ensured to avoid unnecessary burden, including financial and psychological, on patients labeled as celiac.

Current international guidelines emphasize the need for a histological diagnosis after positive serological testing to accurately diagnose CD [4,5]. This method is considered invasive and not without its limitations, as microscopic changes may be non-specific or patchy, resulting in a missed diagnosis of CD [6].

Interestingly, over the past few years more has been published on the possibility of a non-biopsy approach to the diagnosis of CD in adults, after the European Society for Paediatric

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Conflict of Interest: None

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Gastroenterology and Nutrition (ESPGHAN) updated their guidelines for diagnosing CD in children and adolescents to favor this approach in 2020. These guidelines confirmed that a non-biopsy approach is safe in children and adolescents when the immunoglobulin A (IgA) anti-tissue transglutaminase (tTg) antibody value is ≥10 times the upper limit of normal (ULN), in the presence of positive IgA endomysial antibodies (EMA) in a second serum sample. These guidelines highlight that a CD diagnosis can be made without a histological sample, as CD was invariably evident in patients with very high serum celiac auto-antibody levels [7].

In this retrospective study, we investigated whether a non-biopsy approach to the diagnosis of CD can be applied to the adult cohort. The aim of this study was to determine whether having an IgA tTg antibody titer ≥10× the ULN significantly correlates with a histological diagnosis of CD, and thereby eliminates or reduces the need for diagnostic biopsies.

Patients and methods

The study was conducted at Mater Dei Hospital, a large tertiary hospital in Malta. Adult patients (≥16 years) who underwent scheduled upper gastrointestinal endoscopy with duodenal biopsies, recorded as OGD+D2 on the hospital booking system, between 2012 and 2024 were analyzed retrospectively. Patient demographics, symptoms, risk factors, celiac serology (both anti-tTg and EMA) and histological diagnoses were collected and reviewed. The quantity and location of duodenal biopsies were recorded.

During the study period, 3 different kits were found to be used to measure tTg antibodies. The cutoff values for each kit are shown below. Anti-tTg was deemed positive, negative or ≥10 times the ULN, based on the cutoff for the individual test kit, as shown in Table 1.

Details on patients who had a repeat biopsy, in view of positive serology but an initial negative biopsy, were also recorded. Ethical approval was obtained for the study.

Results

In total, 114 patients (78.1% female; mean age 41.0±18.1 years) were included in the study. All patients had normal IgA levels, while 97.4% tested positive for IgA tTg antibodies and 93.8% positive for EMA.

Table 1 Cutoff values of the different immunoglobulin A (IgA) anti-tissue transglutaminase (tTg) kits and number of patients per kit

Kit	Cutoff values	No. of patients
Kit 1 (Orgentec)	U/mL (0-15)	48 (42.1%)
Kit 2 (Euroimmun)	RU/mL (0-19.9)	31 (27.2%)
Kit 3 (Eurospittal)	IU/mL (0.1-9)	35 (30.7%)

Symptoms and histology

Of the above cohort, 73.7% (84/114) exhibited some form of pathological findings on duodenal biopsies, while 26.3% had normal biopsies.

- Normal: 26.3%
- Celiac disease: 70.2%
- Duodenitis: 2.6%
- Focal intraepithelial lymphocytosis: 0.9%

A histological diagnosis of CD was confirmed in 70.2% (80/114). When patients were stratified by sex, 75.3% of females were diagnosed with CD compared to 24.7% who were negative, while 52% of males were diagnosed with CD and 48% were negative. The prevalence of CD was found to be significantly associated with sex (P=0.025).

Marsh subtype classification was available in 95.0% of cases. There was a nearly equal distribution between subtypes 3a (31.6%), 3b (43.4%) and 3c (22.4%). Marsh 1 classification was present in 2.6% of patients. After multidisciplinary discussions between the pathologist and gastroenterologist, these patients were treated as having CD in view of their clinical history and serological markers.

Symptoms suggestive of CD were reported in 79.8% of patients undergoing endoscopy (Table 2), while the remaining 20.2% were asymptomatic and were investigated for other reasons, such as abnormal blood results or screening (Table 3).

Additional autoimmune pathologies were present in 15.7% of patients, these being autoimmune thyroid disease (8.8%) and type 1 diabetes mellitus (6.9%). Approximately a quarter of the patients (24.5%) had a first-degree relative with CD, though only 55.7% of patients had suggested screening to their relatives following diagnosis.

Table 2 Clinical characteristics including presenting symptoms and Marsh histology

Characteristics	Value
n	102
Mean age±SD, years	40.1±17.9
Female	81
Presenting signs and symptoms in patients	%
Bloating	52.0
Change in bowel habit	42.2
Abdominal pain	41.2
Fatigue	31.4
Iron deficiency anemia	24.5
Weight loss	16.7
Dermatitis herpetiformis	12.7
B12 or folate deficiency	10.8
Marsh Scores	% Total n=73
1	2.7
3a	30.1
3b	43.8
3c	23.3

SD, standard deviation

Table 3 Reasons for investigation of asymptomatic patients of all the cohort

Iron deficiency anemia	10.5%
B12 or folate deficiency	10.5%
Type 1 diabetes	15.8%
Autoimmune thyroid disease	10.5%
First degree family member with CD	21.1%

CD, celiac disease

Four patients (3.5%) who had normal tTg levels were referred for duodenal biopsies because of gastrointestinal symptoms. Of these, 3 patients were histologically diagnosed with CD despite having normal antibody levels.

Serological data analysis

Elevated IgA tTg levels were found to be significantly predictive of a histological diagnosis of CD, as demonstrated by the receiver operating characteristic (ROC) curves.

For Kit 1 (U/mL 0-15.0; n=48), the area under the curve (AUC) was 0.86 (95% confidence interval [CI] 0.76-0.98; P=0.002). The level of 30.7 U/mL was identified as the point at which sensitivity and specificity were most favorably balanced, resulting in a sensitivity of 93% and a specificity of 73%. At 62.8 U/mL, sensitivity was 73% and specificity was 72%. When tTg levels exceeded 10 times the ULN, the kit achieved a sensitivity of 61% and a specificity of 100% (Fig. 1).

For Kit 2 (RU/mL 0-19.9; n=31), the AUC was 0.94 (95%CI 0.87-1.00; P<0.001). The optimal balance between sensitivity and specificity occurred at a tTg level of 74.7 RU/mL, corresponding to a sensitivity of 94% and a specificity of 74%; this cutoff is approximately 3.75 times the ULN for this test kit. At ≥10 times the ULN, the kit demonstrated a sensitivity of 69% and a specificity of 100% (Fig. 2).

For Kit 3 (IU/mL 1.0-9.0; n=35), the AUC was 0.82 (95%CI 0.68-0.96; P=0.002). The most favorable sensitivity and specificity pairing was found at a tTg level of 20.2 IU/mL, with a sensitivity of 87% and specificity of 67%. A tTg level of 25.2 IU/mL provided a sensitivity of 78%, while specificity remained at 67% (Fig. 3).

Given the use of 3 different test kits with varying cutoff values, the tTg results were standardized based on the ULN for each kit so that a combined ROC curve analysis could be performed. By adjusting for the cutoff values of the different kits, a standardized tTg result was established, corresponding to the ULN for each kit. The combined analysis yielded an AUC of 0.87 (95%CI 0.79-0.93; P<0.001). At 10 times the ULN, sensitivity was 59% with a specificity of 100%. At 5 times the ULN, sensitivity increased to 74%, with a specificity of 81%.

A total of 41.2% (n=47) of patients exhibited a tTg level ≥10 times the ULN. An IgA tTg level ≥10 times the ULN demonstrated a sensitivity of 58.8% (95%CI 48.5-68.3%) and a specificity of 100% (95%CI 89.7-100%). The positive predictive value was 100% (95%CI 92.4-100%), while the negative predictive value was 50.7% (95%CI 39.0-62.3%; P<0.001). Since all patients with a tTg level ≥10 times the ULN also had positive EMA results, the combined predictive values reflect both tTg ≥10 times the ULN and EMA positivity. No patients with tTg ≥10 times the ULN had a negative EMA result, thus precluding the calculation of sensitivity and specificity for this subset.

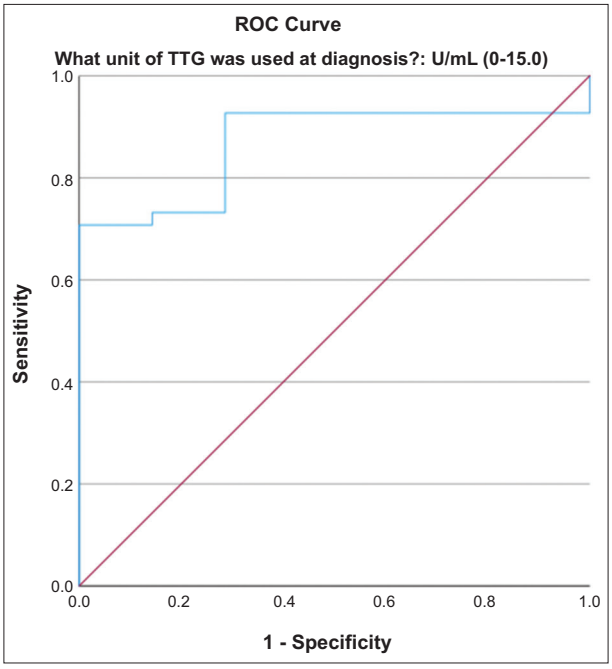

Figure 1 ROC curve analysis for Kit 1 U/mL (0-15.0) n=48, AUC 0.86, 95%CI 0.76-0.98; P=0.002
ROC, receiver operating characteristic; AUC, area under the curve, CI confidence interval

Among patients with a tTg level below 10 times the ULN, 41.3% (n=33) were confirmed positive for CD on biopsy. Seven patients who tested negative for CD on the initial biopsy underwent re-biopsy at least 12 months post-initial endoscopy. None of these re-biopsied patients had a tTg level ≥10 times the ULN, either on initial or repeat serology.

EMA antibody testing was conducted in 99.1% of the cohort, with 93.8% of patients showing positive EMA results. Of those, 98.1% also had positive tTg results (P=0.048). Additionally, 72.6% of patients with EMA positivity were histologically diagnosed with CD (P=0.14). However, 27.4% (n=29) of patients with positive EMA results were not confirmed to have CD on biopsy. Notably, 2 patients were diagnosed with CD despite negative EMA results.

Biopsies from the second part of the duodenum (D2) were obtained in 99.1% of patients, with 85.8% undergoing ≥4 biopsies (mean: 5.25±1.65). Biopsies from the first part of the duodenum (D1) biopsies were collected in 59.6% of patients (mean: 1.23±1.29), while 47.5% of patients had D2 biopsies only, without D1 sampling. A significant concordance was observed between the biopsy results from D1 and D2, for both

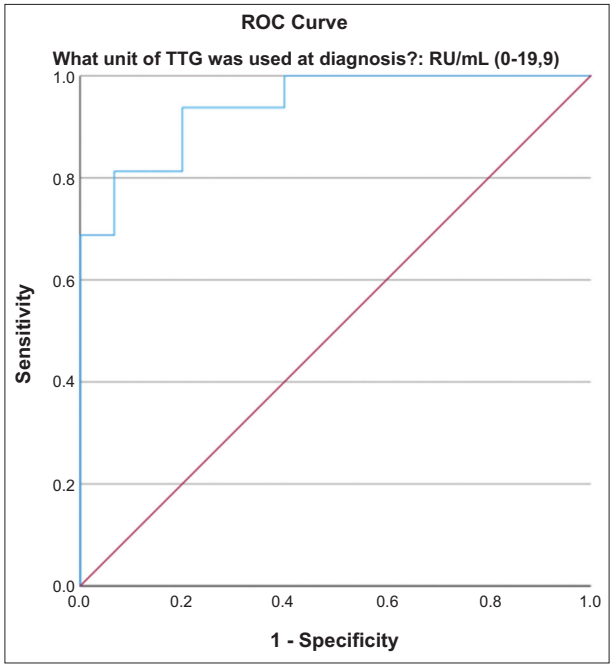

Figure 2 ROC Curve for Kit 2 RU/mL (0-19.9) n=31, AUC 0.94, 95%CI 0.87-1.00; P<0.001
ROC, receiver operating characteristic; AUC, area under the curve, CI confidence interval

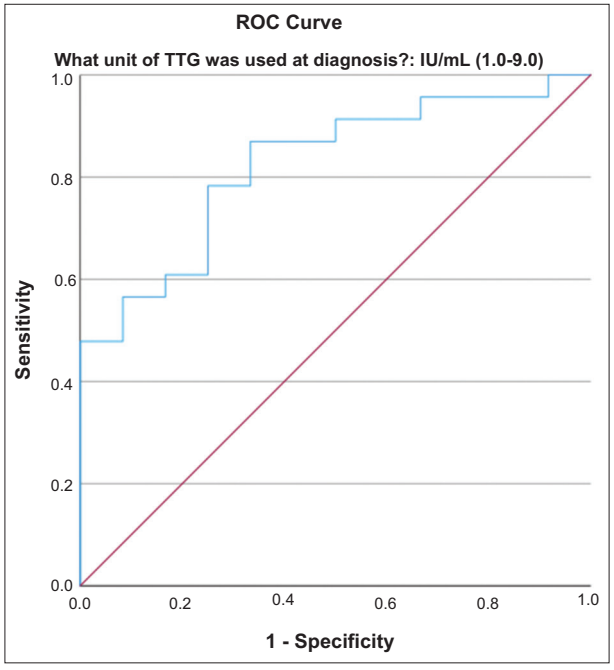

Figure 3 ROC Curve for Kit 3 RU/mL (1-9) n=35, AUC 0.82, 95%CI 0.68-0.96; P=0.002
ROC, receiver operating characteristic; AUC, area under the curve, CI confidence interval

positive and negative findings (P<0.001). Specifically, 91.7% of patients with positive D1 biopsies were also positive for CD on D2 biopsies, and 82.6% of patients with negative D1 biopsies were similarly negative for CD on D2. Notably, 8.3% of diagnosed patients exhibited histological abnormalities solely on D1 biopsies, while 17.4% were diagnosed with CD based solely on D2 biopsies.

Discussion

Villous atrophy observed in small intestinal biopsies was for a long time the mainstay for diagnosing CD in all individuals up until 2020, before the ESPGHAN CD group released guidance on a no-biopsy approach for the pediatric cohort [7,8]. The traditional method involves an invasive procedure, which increases costs and delays in diagnosis [4,5]. Relying on histological diagnosis can result in missed cases of CD, due to non-specific microscopic changes (especially in early disease) and the overall patchy involvement of the small intestine. This dependency may also result in repeat endoscopies in patients with symptoms and serological tests highly suspicious for CD, but with normal histological reports [6].

As a result, over the past decade a no-biopsy approach for the diagnosis of CD in patients who show a 10-fold increase in IgA tTg antibody levels has garnered significant interest [3,5,6,9], especially since ESPGHAN published guidelines recommending this approach, in the presence of a positive EMA test, for the pediatric cohort in 2020 [7]. Moreover, the British Society of Gastroenterology (BSG) also published interim guidance during the COVID-19 pandemic, endorsing this strategy [10]. However, this approach has not yet been widely adopted in adults and is only incorporated into the Finnish guidelines for diagnosing CD in adults [11]. Overall, it remains highly controversial, and further studies are required to confirm the accuracy of a no-biopsy approach before it can become widely established practice.

Several studies have demonstrated that reliance on serology may be safe in the adult cohort [12-14]. Recently, a large systematic review and meta-analysis concluded that patients with a high pretest probability of CD and an IgA tTg ≥10 times the ULN could be diagnosed as having CD without duodenal biopsies. This conclusion was based on the finding that an IgA tTg level ≥10 × ULN has 100% specificity and a positive predictive value of 98% [5]. These findings align with our results, which further corroborate this conclusion.

EMA testing increases the predictive accuracy of a non-biopsy approach, and is recommended in the ESPGHAN guidelines [7]. Our study also supports this, as all the patients with an IgA tTg level ≥10 × ULN had a positive EMA result, and none of the patients with a negative EMA were diagnosed with CD on biopsy. However, EMA testing increases costs and labor, and many laboratories have discontinued this test, limiting its applicability worldwide [5].

The importance of obtaining both D1 and D2 biopsies in patients suspected of having CD is highlighted in this study: 8.3% of patients with normal D2 biopsies were

diagnosed with CD based on Marsh 3 changes identified in concurrently obtained D1 biopsies. This underscores the need to collect multiple biopsies from both D1 and D2 to improve diagnostic accuracy, as recommended by the BSG guidelines in 2014 [15]. It is also important to note that patients with negative biopsies for CD, despite high IgA tTg antibody levels, may require re-biopsy. In our study, 75% of our patients with an initial negative biopsy were later diagnosed with CD upon re-biopsy. These results may reflect the patchy nature of small bowel changes in CD and the difficulties in interpreting certain microscopic changes.

The limitations of this study include its retrospective nature and the fact that it originates from a single center. Another limitation is that all the patients had a high pretest probability of CD, which may reduce the utility of this approach in primary care settings, where pretest probability is lower. Additionally, 3 different kits were used for anti-tTg antibody testing, each with different cutoff limits, complicating the analysis. However, it delivers important information on the use of different kits in such analysis and provides information in different population cohorts.

Strong arguments against a no-biopsy approach exist, primarily focusing on unreliable IgA tTg kits [15], the risk of missing concomitant diseases [16] and the necessity of a definitive diagnosis of CD, as patients must adhere to a lifelong gluten-free diet, which presents its own challenges.

On the other hand, with growing evidence that an IgA tTg antibody titer ≥10 times the ULN is 100% specific for diagnosing CD, a no-biopsy approach could reduce delays in diagnosis, minimize invasive procedures, and lower healthcare expenses. In fact, patients have been shown to prefer a no-biopsy approach, which highlights the need for shared decision-making and patient-centered care [1,4,5], particularly until official guidance regarding a no-biopsy approach is published. Moreover, patients with persistently positive celiac serology, especially if ≥10 times the ULN, and negative duodenal biopsies, despite multiple biopsies, should be considered for repeated small intestinal biopsies and possible small bowel capsule imaging, as given the sensitivity and specificity of IgA tTg at this level, as demonstrated in this study, there is a significant risk of missing a CD diagnosis.

In conclusion, the findings of this retrospective study support the growing evidence that a biopsy-free approach may be appropriate in diagnosing CD when IgA tTg levels are ≥10 times ULN, especially in the presence of a typical clinical picture, risk factors, EMA positivity and patient preference. Future studies could focus on testing patients in primary care settings with a low pretest probability of CD and determining which commercial IgA tTg kits are reliable for widespread use in all centers globally.

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Summary Box

What is already known:

- Standard practice for diagnosing celiac disease involves duodenal biopsies and histological examination
- A biopsy-free approach for diagnosing celiac disease in pediatric patients is well-established
- Patchy involvement of the small bowel in celiac disease may result in false negative histological findings

What the new findings are:

- This study further supports recent findings that a biopsy-free approach may be appropriate for diagnosing celiac disease when immunoglobulin A (IgA) anti-tissue transglutaminase (tTg) levels are ≥10 times the upper limit of normal
- An IgA tTg level ≥10 times the upper limit of normal showed a positive predictive value of 100% and a negative predictive value of 50.7% (P<0.001)
- The standardization of IgA tTg assay kits can enhance the reliability of adopting a widespread biopsy-free diagnostic approach for celiac disease

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